FEEDING MECHANISMS AND NUTRITION IN THREE SPECIES OF BRESSLAUA

C. LLOYD CLAFF, VIRGINIA C. DEWEY AND G. W. KIDDER

(From the Arnold Biological Laboratory, Brown University, and the Marine Biological Laboratory, Woods Hole, Massachusetts)

The question of food taking by protozoa has attracted considerable attention in the past and there have appeared numerous accounts of the various mechanisms employed, the type of food taken and the conditions of acidity and alkalinity during the digestive process. Regarding the last-mentioned observations there seems to be general agreement that, in the bacteria-feeding species at least, there is an acid-alkaline cycle from the time the food is ingested until the residue is defecated. Practically all of the observers employed some type or combination of types of indicator dyes, watching for the color changes which occur as the food is ingested, digested and the residue defecated. The most frequently used indicator has been neutral red because of the ease with which most protozoa take up this dye. Unfortunately, this indicator is useful only to detect shifts in hydrogen ion concentrations through a relatively small range.

We have examined the problem of feeding and acid-alkaline reactions in three species of the genus *Bresslaua.* These ciliates are carnivorous members of the family Colpodidae and one species, due to its peculiar feeding habits, offers exceptional opportunities for direct observations for long, uninterrupted periods of time.

In the ensuing account we will give a short description of the experimental organisms, an account of their feeding habits, some evidence for an acid-alkaline cycle during digestion and a brief account of food selectivity.

Material and Methods

The carnivorous ciliates were obtained from dry hay collected in Stuart, Florida. The same procedures of excystation and isolation were employed as were previously used in the case of most of the *Colpoda* material reported from this laboratory (Kidder and Claff, 1938; Kidder and Stuart, 1939; Burt, 1940).

For studies on the hydrogen ion concentration within the vacuoles

and the protoplasm various indicator dyes were used. These will be described in a later section. It was found expedient first to stain the food organisms (usually *Colpoda steinii*) and then to add a few *Bresslaua* to the culture. The culture was then placed in a moist chamber until the food organisms had all been ingested. As in the case of *Woodruffia metabolica* (Johnson and Evans, 1939; 1940), these carnivores formed resistant cysts after the food had become depleted. These were caused to excyst by the addition of fresh hay infusion. Food organisms were then added and the feeding process studied under a water immersion lens (× 40). The dye brought into the protoplasm of the carnivores during the previous period of feeding was sufficient to allow us to gain an idea of the changes in acidity and alkalinity which took place during feeding, digestion and the subsequent defecation of residue.

For the study of food selectivity bacteria-free ciliates were necessary. The *Bresslaua* were freed of their associated microörganisms by the employment of our modification of the Parpart method of direct washing, using Syracuse watch glasses enclosed in cellophane bags (Kidder, Lilly and Claff, 1940). Because of the structural peculiarities of these ciliates it was found necessary to allow them to encyst and divide after the tenth wash in sterile hay infusion. Close watch was kept of the dividing ciliate so that the washing could be continued immediately after the emergence of the daughter organisms. Each of the two, four or more daughters was then washed individually through five or more changes of sterile medium and placed in tubes containing the food organism to be tested. Adequate bacteriological tests showed that the majority of the carnivores so treated were free of bacteria.

The food organisms tested will be discussed in a later section. They have all been mentioned in previous accounts from this laboratory.

Description of Bresslaua Kahl

The three members of this genus which we have studied resemble the various species of *Colpoda* in their general structure, mode of division within a cyst and permanent cyst formation. They all possess a macronucleus of the *Colpoda cucullus* type (Kidder and Claff, 1938; Burt, 1940) and a single micronucleus. The chief differences are found in the structure of the mouth, which has become modified and extended for the carnivorous mode of life. The following brief descriptions are given to add to the account of Kahl (1931) of *B. vorax* and to establish two new species.

Bresslaua vorax Kahl (*Fig. 1, A*).—This species is evenly rounded posteriorly, but the anterior end is compressed laterally. The left an-

terior side is depressed in such a way that the whole anterior end is twisted. This twisted appearance is seen best in an organism immediately after excystment. The size varies greatly depending upon the amount and kind of food taken. Freshly excysted ciliates range in length from 40 μ to 90 μ and in width from 25 μ to 50 μ. Ciliates which have fed on relatively large prey (such as *Glaucoma scintillans* or *Colpoda cucullus*) attain a size of 180 $\mu \times 100\ \mu$ or even larger.

The ciliary pattern, as seen after the silver technique of Klein or when treated with opal blue or nigrosin, resembles that of other members of the family. The peripheral cilia arise in pairs, as is true of most of the cilia among the members of the genus *Colpoda* (Taylor and Furgason, 1938; Burt, 1940). This is in contrast to the condition in *Woodruffiia metabolica* (Johnson and Larson, 1938) where the cilia are single. The cilia are relatively short and delicate. The ciliary rows originate from a short keel and extend over the general body surface as well as the right interior of the cytostomal cavity, converging in a field at the posterior end of the body.

The mouth is a large, cilia-lined cavity, open toward the ventral surface and the left side. On the roof of the mouth are folds or "rugae," roughly resembling those on the hard palate of mammals. On the floor of the mouth, which is somewhat raised, there is a row of membranelle-like structures, 40 to 45 in number. These beat in such a way as to create a strong current *out* of the mouth. At the back of the mouth there is a rather short, broad gullet directed posteriorly. It is on the brink of this gullet that the membranelles are located.

Bresslaua vorax exhibits activity when not actually feeding. It tends to remain on or near the bottom of the culture and to move in small circles. It comes in contact with the bottom so that the left side of the body, and therefore the mouth-opening, is up. Prey are swept into the mouth by strong currents. During the time the live prey is in the mouth until it has entered the food vacuole at the base of the gullet the movement of the carnivore is much reduced. This is due to a change in the beating of all the peripheral cilia and will be described in greater detail in the case of one of the other species. After the prey has been successfully trapped in the posterior food vacuole, movement is resumed.

Bresslaua insidiatrix sp. nov. (*Fig. 1, B*).—The general departures from the *Colpoda*-like structure which were described for *B. vorax* are accentuated in this species. The mouth opening is more extensive in relation to the size of the body and the twisting of the anterior end is somewhat greater. No "rugae" are present in the mouth. This species varies in size from 40 $\mu \times 25\ \mu$ when starved to 120 $\mu \times 90\ \mu$ when

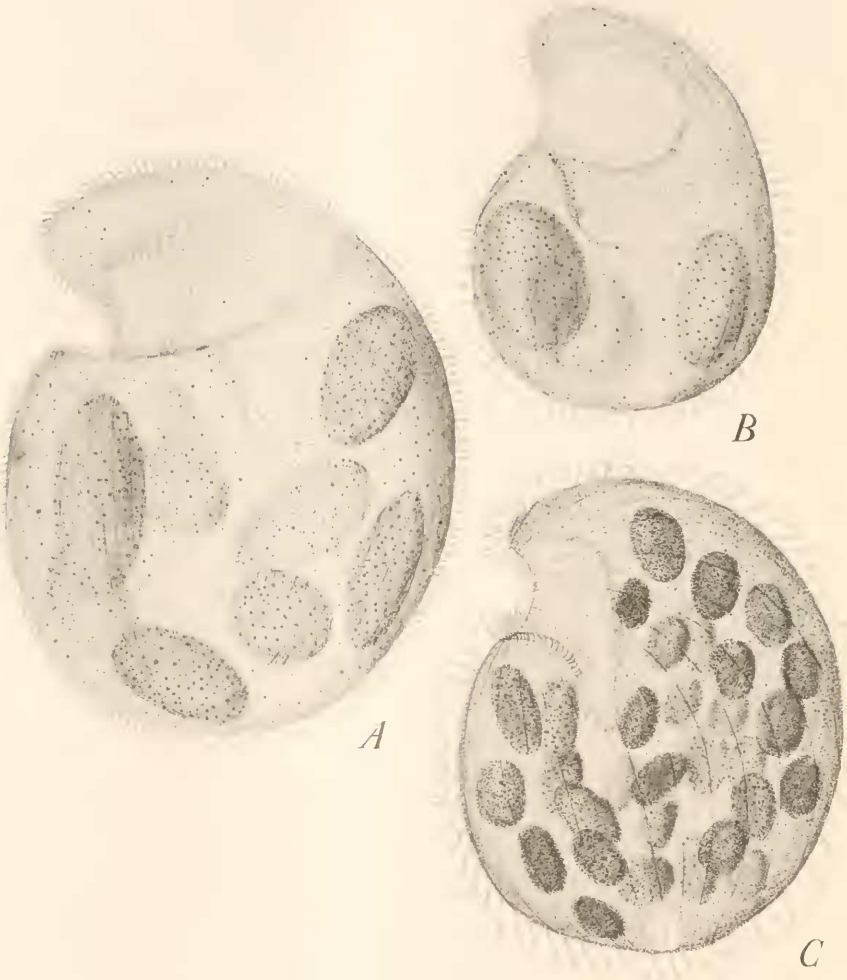

FIG. 1. All drawings were taken from life. × 460. *A. Bresslaua vorax.* The food inclusions are *Glaucoma scintillans. B. Bresslaua insidiatrix* sp. nov. during early stages of feeding on *Glaucoma scintillans. C. Bresslaua sicaria* sp. nov. after ingesting a number of *Colpoda steinii.*

ready to divide after active feeding. The general pattern of the peripheral ciliary lines is similar to that in *B. vorax*. The cilia originate in pairs and are very long and stiff. They are easily visible in life and stand out at nearly right angles to the body while the organism is at rest. There are 10 to 15 membranelle-like structures in the mouth located in the same relative position as those in *B. vorax*.

One of the most characteristic things about *Bresslaua insidiatrix* is its mode of feeding. It normally rests on the bottom of the culture dish with its right anterior end in contact with the substratum. It will remain for two to three hours in one spot, only occasionally pivoting slightly. During this time there is a strong current being directed into the very large mouth-opening and all small objects are drawn in. Inanimate objects are rapidly whirled toward the posterior border of the mouth and shot out by means of the out-going current created by the membranelles. Moving ciliates or flagellates, on the other hand, receive different treatment. Some mechanism within the mouth seems to be stimulated by ciliated or flagellated organisms and this appears to affect the whole neuromotor system. The peripheral cilia immediately lose their stiff, vibratile appearance and move slowly in waves (Fig. 2, *B, C*). The current going into the mouth slackens or disappears as does the outgoing current along the posterior border. The mouth-opening is contracted, forming an efficient barrier against the escape of the prey. The prey moves about freely in the mouth for from one to two minutes and gradually the posterior border of the mouth begins to form the prospective food vacuole. This vacuole forms well ahead of the prey and not under direct impact of it. The prey may partially enter the forming food vacuole and draw back into the mouth a number of times before it is finally trapped. Once well within the vacuole it begins to rotate and the vacuole closes off. The closure is effected by what appears to be a thin sheet of protoplasm originating from the region just posterior to the zone of membranelles and flowing across the vacuole opening from ventral to dorsal. At the instant the sheet of protoplasm fuses with the opposite side the prey is killed. This phenomenon will be discussed later in the section on hydrogen ion concentrations. The closure of the vacuole also sets off another reaction which immediately causes the peripheral cilia to resume their stiff, vibratile condition (Fig. 2, *D*).

Bresslaua insidiatrix appears to be the most highly specialized for a carnivorous habit of the three species observed by us. It feeds only on living ciliates and flagellates. Other bodies (cysts, amoebae, algae, yeast and detritus) do not evoke the "swallowing" response. That this evocation is largely physical is indicated by the following fact. In an excysting culture of *Colpoda steinii* it is common to see these small

ciliates rotating rapidly within the thin endocyst. These ciliates may be drawn into and swept out of the mouth of *B. insidiatrix* a number of times while the endocyst is still intact, but immediately the *Colpoda* escapes its cyst wall and is drawn into the mouth, it evokes the general responses noted above. In contrast to this, both *Bresslaua vorax* and the third species, yet to be described, are able to ingest certain types of non-moving microörganisms, but not all organisms ingested are adequate as food.

Bresslaua sicaria sp. nov. (*Fig. 1, C*).—This species shows a closer resemblance to the typical *Colpoda*-form than either of the above-mentioned species. The mouth opening is confined to the ventral surface and does not extend to the left side. The interior of the mouth cavity is similar in structure and relative size to that of *B. vorax,* but lacks "rugae." The zone of membranelle-like structures is composed of from 20 to 25 components and occupies the same general position as that in the preceding species. A well-formed gullet is present running posteriorly a short distance into the cell.

Bresslaua sicaria varies from 35 μ to 110 μ in length and from 23 μ to 92 μ in width depending upon its state of nutrition. The peripheral ciliary lines are less numerous than those of the other two species, but the general patterns are very similar. The cilia are long and wavy and originate in pairs.

Bresslaua sicaria, unlike the other two species, rarely comes to rest. It swims in a characteristic looping fashion and draws its prey into the mouth while swimming. There is a change in the ciliary motion during the act of swallowing resulting in general and violent movement of the whole organism. Immediately a food organism is caught the *Bresslaua* starts rotating rapidly on its lateral axis and continues the rotation until the prey enters the vacuole, when it resumes its swimming motion. The feeding reactions of this species are very difficult to observe because of its extreme activity.

The feeding habits of the three species described above are so characteristic that it is possible to identify each of them under very low magnifications. *Bresslaua vorax* and *B. insidiatrix* take their prey while they are in contact with the solid substratum, while *B. sicaria* feeds while swimming free in the medium. Of the first two, only *Bresslaua insidiatrix* remains motionless while waiting for its prey. Because of this characteristic, *B. insidiatrix* is an ideal carnivore to use in experiments and observations on feeding mechanisms.

The establishment of two new species of the genus *Bresslaua* seems to us to be justified because of the characteristics noted above (number of

ciliary rows, length and characteristics of cilia, shape and extent of cytostomal opening, feeding habits and food selectivity).

Food Vacuoles and Hydrogen Ion Concentration

After *Bresslaua insidiatrix* has fed on *Colpoda steinii* previously stained with a 1:12 million dilution of neutral red, it becomes highly colored by virtue of its food inclusions. After the food has been exhausted the carnivores form protective cysts. Many red food balls are still present in the encysted organisms. These food balls are defecated during or shortly following excystment (Fig. 2, *A*, *B*), leaving the ciliates nearly colorless. Under the water immersion lens it is possible to detect a number of neutral red stained granules in the endoplasm. Excystment with alkaline hay infusion imparts a slight yellowish tinge to the medium, but does not change the color of the endoplasmic granules. The small freshly excysted ciliates settle to the bottom of the culture dish and immediately begin feeding when numbers of *Colpoda steinii* are added with the excysting fluid (Fig. 2, *B*). The clearest observations are made during the capturing and killing of the first several ciliates.

As the prospective food vacuole forms its fluid contents become slightly pink (Fig. 2, *C*). This coloration deepens as the prey enters, but there appears to be no change in the motions of the prey at this time. At the instant that the food vacuole is closed off by the protoplasmic sheet there suddenly appear a large number of brilliant red granules or droplets in the protoplasm surrounding the vacuole (Fig. 2, *D*). The fluid surrounding the prey then becomes more deeply colored and simultaneously the prey is killed. The prey becomes motionless and the cilia stand out from the body. The fluid rapidly disappears from the vacuole and its lining comes to lie very close to the prey. The red granules in the cytoplasm rapidly fade out. There appears to be no indication that they enter the vacuole as has been described by Nirenstein (1905) for *Paramecium*. This is the first color-change to be noted. The reaction with neutral red shows that an acid condition is suddenly attained and that the hydrogen ion concentration is equal to or less than a pH of 6.8.

The above observations were repeated a number of times using a number of indicator dyes. None of them was quite as spectacular as the neutral red, either because they did not penetrate or because the colors were more difficult to see. Methyl red, methyl orange, brom cresol green, brom phenol blue, brom phenol purple, chlor phenol red, para-dimethyl-amino-azobenzene (Töpfer's reagent), Congo red and benzene-azo-alpha-naphthylamine were used and of these methyl red was by far the best. Although not as brilliant as the neutral red reaction, all of

the phases appeared with this dye. The appearance of bright red granules with methyl red indicates that their acidity must be in the neighborhood of pH 4.2 or lower. Failure of blue coloration with Congo red indicates that the hydrogen ion concentration is probably not higher than pH 3.0.

It appears likely that the sudden death of the prey is the result of the release of an acid from the protoplasm of the carnivore into the vacuole. Töpfer's reagent failed to give positive results in this organism, although the dye penetrated well. Nirenstein (1905; 1925) had reported using this dye to detect the presence of mineral acid in the vacuole of *Paramecium*, as indicated by the appearance of a red color. No red coloration was obtained in *Bresslaua*. Just what type of acid is released is obscure.

Separate experiments show that the acidity indicated by the color changes with methyl red are compatible with the death points of the various types of prey. Thus, *Colpoda steinii* is killed almost instantly in a phosphate buffer of pH 3.8, while the *Bresslaua* is still alive after one hour at pH 3.4. *C. steinii* died only after long periods at pH 4.5 and above this value no death was observed. This experiment simply shows that *Bresslaua* is more resistant to high acidity than is *Colpoda* and lends support to the idea that the killing within the vacuole is a result of the release of acid. A similar conclusion regarding the function of acid was reached recently by Mast and Bowen (1940) in the case of *Vorticella*. Other food organisms which were tested were more resistant than *Colpoda*. *Euglena gracilis and Astasia klebsii* survived for a long time at pH 3.8 and this checks with the reactions of these two flagellates within the food vacuole of *Bresslaua*. After the protoplasmic sheet has closed over either of these organisms there elapses from two to ten minutes before euglenoid movement ceases.

Following the killing process the body of the *Colpoda* begins to move anteriorly due to the general cyclosis of the protoplasm of the *Bresslaua*. When the prey contains an indicator dye, such as neutral red, it is possible to follow the color changes occurring during the hour or two required for digestion. At first the prey is nearly colorless, but it rapidly becomes yellowish. This indicates a faintly alkaline reaction and corresponds to the situation found in *Paramecium* (Nirenstein, 1925) except that the vacuole has never been observed to swell. The yellow color remains for from 15 to 20 minutes and then gradually changes through orange to a bright cherry red (Fig. 2, *E*). By the time the prey has reached the red condition its general outline is lost and it has become a compact ball. A number of these balls later fuse and form the fecal mass which is extruded during or following the next encystment, either

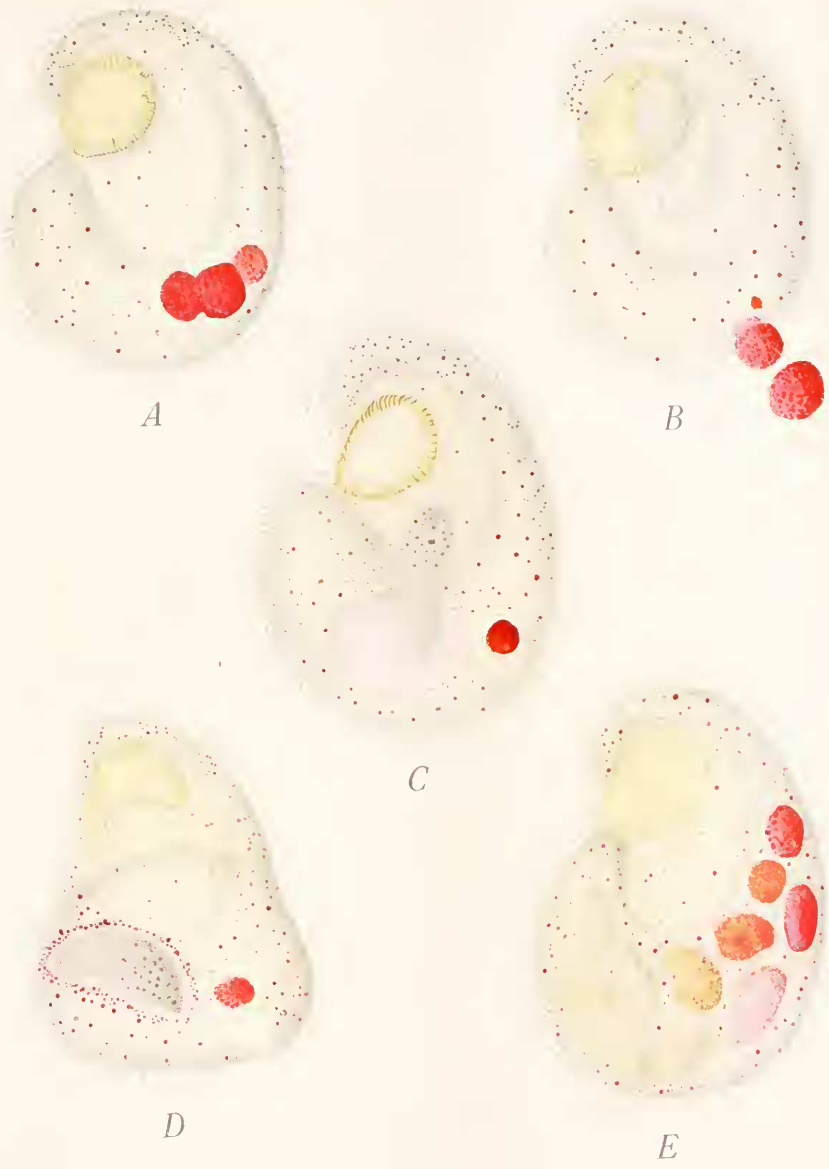

FIG. 2. *Bresslaua insidiatrix* showing the color changes with neutral red during feeding. *A*. Freshly excysted ciliate with old residue. Note the position of the cilia. *B*. Trapping of prey, *Colpoda steinii*, and defecation of residue. The cilia are bent and move in slow waves during this stage. *C*. Prey entering the prospective food vacuole, the fluid content of which is a faint pink. *D*. Food vacuole closed off from the mouth. At this stage the prey is instantly killed. Note the appearance of the cherry red granules in the cytoplasm surrounding the vacuole. The peripheral cilia of the carnivore have again assumed a stiff appearance. *E*. Carnivore after having ingested a number of ciliates. Note the color changes in the bodies of the prey as digestion proceeds.

from a division cyst or a protective cyst. Once released into the surrounding medium the fecal masses rapidly lose their red color, become pale yellow and disintegrate.

As mentioned previously, these observations are best made with *Bresslaua insidiatrix,* because of its feeding habits. As far as could be detected, the same general phenomena take place in the other two species. Certainly the color changes during digestion and defecation are the same, but the color changes accompanying killing, being of such short duration, could never be definitely established due to the constant movement of the carnivores.

Food Selectivity

The following account of the food selectivity is based on our observations of the three species of *Bresslaua* in the presence of a mixed flora of bacteria and in bacteria-free culture. While these observations do not represent a complete survey of the possibilities, they are presented in order to indicate the differences between the species and the possibilities for future work. In Table I we have listed the various food organisms which were used and have summarized the pertinent observations. It will be noted that ingestion does not invariably mean that the organism in question represents adequate food for growth. The various species of *Colpoda* supported growth in all three species of *Bresslaua* and these ciliates probably represent their natural food. The very nature of their protective cyst formation makes this assumption plausible. When dry hay is placed in spring water the various species of *Colpoda* excyst first, feed and multiply before the *Bresslaua* excyst. This means that in nature there would usually be a source of *Colpoda* at the right time.

Glaucoma scintillans was ingested by *Bresslaua vorax* and *B. insidiatrix,* while in the case of *B. sicaria* this was never observed. Thriving bacteria-free cultures of *B. vorax* and *B. insidiatrix* were maintained for a number of months with *G. scintillans* as food. In neither case, however, were normal protective cysts formed. After the food organisms had all been ingested the carnivores continued in the trophic condition for many days, getting smaller and smaller. Occasionally, in the case of *B. vorax,* they would round up and form temporary cysts (Johnson and Evans, 1940) from which they would spontaneously excyst within a few hours. This process might be repeated for days until finally all of the carnivores were dead. Serial transplants were always made while some food was still present. Eventually the various series declined in division rate and failed in transfer. The causes associated with this decline must receive further investigation.

TABLE I

Hay = sterilized hay infusion; *V* = *Bresslaua vorax*, *I* = *B. insidiatrix* sp. nov.; *S* = *B. sicaria* sp. nov.; (*N*) = non-sterile; (*S*) = sterile; (*S**) = no bacteria present but *Colpoda* were growing on *Stichococcus;* 1, 2, 3, 4, = relative growth where 4 is maximum observed. Where blanks occur in columns labeled "Ingestion" and "Growth" those organisms were not used.

Food Organism	Medium	Ingestion			Growth			Remarks		
		V	*I*	*S*	*V*	*I*	*S*	*V*	*I*	*S*
Colpoda cucullus....	Hay, H_2O	+	+	+	1	2	2	Large, normal (*N*)	Very large, normal (*N*)	Large, normal (*N*)
C. steinii.....	"	+	+	+	4	4	4	Medium, normal (*S*N*)	Medium, normal (*S*N*)	Medium, normal (*S*N*)
C. maupasi.....	"	+	+	+	4	2	3	Medium, normal (*N*)	Medium, normal (*N*)	Medium, normal (*N*)
C. aspera.....	"	+	+	+	1	4	1	Small, cysts with low viability (*N*)	Small, normal (*N*)	Small, cysts with low viability (*N*)
C. inflata.....	"	+	+	+	1	3	1	Small, cysts with low viability (*N*)	Small, normal (*N*)	Small, cysts with low viability (*N*)
Glaucoma scintillans	Yeast-Harris, H_2O	+	+	−	3	2	0	Very large, normal (*SN*)	Large, normal (*SN*)	(*SN*)
Tetrahymena geleii..	Hay, H_2O	+	+	+	1	0	0	Very large, abnormal. Cysts non-viable (*SN*)	Toxic (*SN*)	Toxic (*SN*)
Stichococcus bacillaris.....	"	+	−	+	3	0	0	Small, normal (*SN*)	(*SN*)	Encystment without division. (*SN*) Non-viable
Chlorella.....	"	+	−	+	2	0	0	Small, normal (*SN*)	(*SN*)	Encystment without division. (*N*) Non-viable
Euglena gracilis....	"	+	+		0	0		Encystment without division. Non-viable (*N*)	Encystment without division. (*N*) Non-viable	
Astasia klebsii.....	"	+	+		0	0		Encystment without division. Non-viable (*N*)	Encystment without division. (*N*) Non-viable	
Chilomonas paramecium.....	"	+	+		0	0		Encystment without division. (*SN*) Non-viable	Encystment without division. (*SN*) Non-viable	
Yeast (live).....	H_2O	+	−	+	3	0	3	Small, normal (*SN*)	(*SN*)	Small, normal (*N*)
Aerobacter.....	"	+	−	−	1	0	0	Small, normal (*N*)	(*N*)	(*N*)
Unknown bacteria..	"	+			3			Small, normal (*S*)		

Tetrahymena geleii was ingested by all three species but did not support continued growth in any case, although a few divisions of *Bresslaua vorax* usually resulted. *Tetrahymena* appears to be toxic to *B. insidiatrix,* for after a single organism had been ingested the carnivore would leave the bottom of the dish, swim rapidly for a few minutes and then round up and encyst. These cysts were never viable. *Bresslaua sicaria* behaved in a similar manner.

One other item worthy of note in these investigations on nutrition is the case of *Stichococcus bacillaris. Bresslaua vorax* readily ingests this alga and flourishing cultures result. Normal protective cysts are formed and may be collected, dried and stored for future use. By use of the glass plunger-sponge method (Kidder, 1941) any number of sterile carnivores may be kept on hand.

Discussion

While the work on the bacteria-free cultures has not progressed to a point where the nutritional requirements of the members of the genus *Bresslaua* can be stated definitely, a number of points of interest have come to light. One of the most interesting observations is the great difference in the food organisms as evidenced by the differences in nutritional quality between *Glaucoma scintillans, Tetrahymena geleii* and the various species of *Colpoda* for *Bresslaua. Colpoda* was utilized by all three species of *Bresslaua. Glaucoma* was utilized by *Bresslaua vorax* and *B. insidiatrix,* while *Tetrahymena* was utilized by only *B. vorax* and then the growth was poor and not transplantable. This is exactly the reverse of the situation with the carnivorous hypotrichs, *Stylonychia* and *Pleurotricha.* Lilly (1942) has shown that these ciliates will feed and reproduce on *Tetrahymena* but not on *Glaucoma* and *Colpoda.* It is becoming apparent that the exact nutritional requirements of carnivorous ciliates are delicately adjusted and this fact may be of use in the future for comparisons between food organisms.

Regarding our observations on the hydrogen ion changes during swallowing, killing, digestion and defecation, a few comparisons with reports of other workers may be noted. Prowazek (1898) described neutral red granules around the periphery of food vacuoles of *Paramecium.* He supposed that these granules might be the carriers of the digestive enzymes. An alkaline stage during the digestive process was not described. The work of Nirenstein (1905; 1925) was the most complete on this subject. He describes an initial acid phase in the newly-formed food vacuoles of *Paramecium,* the pH being equal to that of a 0.3 per cent solution of HCl. These vacuoles were much more acid than com-

parable ones in a number of other ciliates. After the initial acid stage the food vacuoles increased in volume and became alkaline. Nirenstein believed that digestion occurs only at this stage, the digestive enzymes being trypsin-like in nature. It had earlier been proposed by Hemmeter (1896) that the appearance of an acid phase was the response to living prey and that the acid served as a killing agent. This contention was denied by Métalnikow (1912) because he was unable to demonstrate any regularity in the acid production even in the event that living prey were ingested. In our work on *Bresslaua insidiatrix* the conclusion was reached that the initial acid production around the vacuole was stimulated by the closure of the vacuole and that it was probably this acid which caused the death of the prey. The prey did not become acid in its reaction, however, which may have been due to the combination of the acid with its proteins. Later, enough alkaline material was taken up to cause the protoplasm of the prey to give an alkaline reaction. This alkaline phase appears to be the phase of active digestion, indicating, therefore, that the enzymes involved are catheptic in nature. Before defecation the residue becomes acid, possibly due to the acidic properties of some of the products of digestion. The appearance of a final acid stage in the food vacuole in *Bresslaua* seems to differ from the condition in *Paramecium*. In the latter organism the residue remains alkaline (Shapiro, 1927).

Up to the present time most of the observations on the hydrogen ion concentration of food vacuoles have been confined to bacteria-feeding ciliates. It will be interesting to see if the conditions described above will be found in other carnivorous types.

Summary

1. Three species of *Bresslaua, B. vorax* Kahl, *B. insidiatrix* sp. nov. and *B. sicaria* sp. nov. are described.

2. These ciliates are carnivorous and feed on other small ciliates, members of the genus *Colpoda* being especially favorable as food.

3. Using indicator dyes it was found that the prey is killed simultaneously with a sudden release of an acid into the newly-formed food vacuole. The hydrogen ion concentration of the vacuole fluid was estimated to be between pH 3.0 and pH 4.2. This range includes the death point of various species of *Colpoda*. During digestion the protoplasm of the prey becomes alkaline. The undigested residue becomes acid before defecation.

4. Bacteria-free *Bresslaua* were tested with a number of food organisms and a preliminary survey of their food requirements recorded.

LITERATURE CITED

Burt, R. L., 1940. Specific analysis of the genus Colpoda with special reference to the standardization of experimental material. *Trans. Am. Mic. Soc.*, **59**: 414–432.

Hemmeter, J. C., 1896. On the rôle of acid in the digestion of certain rhizopods. *Am. Nat.*, **30**: 619–625.

Johnson, W. H., and F. R. Evans, 1939. A study of encystment in the ciliate, Woodruffia metabolica. *Arch. f. Protist.*, **92**: 91–116.

Johnson, W. H., and F. R. Evans, 1940. Environmental factors affecting cystment in Woodruffia metabolica. *Physiol. Zoöl.*, **13**: 102–121.

Johnson, W. H., and Enid Larson, 1938. Studies on the morphology and life history of Woodruffia metabolica, nov. sp. *Arch. f. Protist.*, **90**: 383–392.

Kahl, A., 1931. Urtiere oder Protozoa. I. Wimpertiere oder Ciliata (Infusoria). In: Dahl's Tierwelt Deutschlands, Teil 21. Fischer, Jena.

Kidder, G. W., 1941. The technique and significance of control in protozoan culture. In: Protozoa in Biological Research. Ed. G. N. Calkins and F. M. Summers. Columbia University Press, New York.

Kidder, G. W., and C. L. Claff, 1938. Cytological investigations of Colpoda cucullus. *Biol. Bull.*, **74**: 178–197.

Kidder, G. W., D. M. Lilly and C. L. Claff, 1940. Growth studies on ciliates. IV. The influence of food on the structure and growth of Glaucoma vorax sp. nov. *Biol. Bull.*, **78**: 9–23.

Kidder, G. W., and C. A. Stuart, 1939. Growth studies on ciliates. I. The rôle of bacteria in the growth and reproduction of Colpoda. *Physiol. Zoöl.*, **12**: 329–340.

Lilly, D. M., 1942. Nutritional and supplementary factors in the growth of carnivorous ciliates. *Physiol. Zoöl.* (in press).

Mast, S. O., and W. J. Bowen, 1940. The hydrogen ion and the osmotic concentrations of the cytoplasm in Vorticella similis (Stokes) as indicated by observations on the food vacuoles. *Biol. Bull.*, **79**: 351 (Abstract).

Métalnikow, S., 1912. Contributions à l'étude de la digestion intracellulaire chez les protozoaires. *Arch. Zool. expér. et gén.*, 5e ser., **9**: 373–499.

Nirenstein, E., 1905. Beiträge zur Ernährungsphysiologie der Protisten. *Zeitschr. f. allg. Physiol.*, **5**: 435–510.

Nirenstein, E., 1925. Über die Natur und Stärke der Saureabscheidung in den Nahrungsvacuolen von Paramecium caudatum. *Zeitschr. f. wissens. Zool.*, **125**: 513–518.

Prowazek, S., 1898. Vitalfärbungen mit Neutralroth an Protozoen. *Zeitschr. f. wissens. Zool.*, **63**: 187–194.

Shapiro, N. H., 1927. The cycle of hydrogen-ion concentration in the food vacuoles of Paramecium, Vorticella and Stylonychia. *Trans. Am. Mic. Soc.*, **46**: 45–50.

Taylor, C. V., and W. H. Furgason, 1938. Structural analysis of Colpoda duodenaria sp. nov. *Arch. f. Protist.*, **90**: 320–339.